Agglutination Quantification Protocol

1. Grow a 5mL *E. coli* culture overnight.
2. In the morning, add 5 mL sterile LB to make a 1:2 dilution with a total volume of 10 mL.
3. Label 4 eppies A, B, C, and D.
4. Add 600 uL of the diluted liquid culture and 600 uL of 5% BSA in PBS to each eppie.
5. Add 6uL of *E. coli* antibody to eppie B to create a 1:200 antibody dilution.
6. Add 0.5 uL *E. coli* antibody to eppie C to create a 1:2400 antibody dilution.
7. Add 6 uL of non-specific antibody to eppie D to create a 1:200 antibody dilution.
8. Pour samples into 4 cuvettes. Measure initial OD of all samples.
9. Pour samples back into their original eppies. Put all eppies on the rotator for one hour.
10. After one hour, split the volume in each eppie among two 30 micron spin columns. There should be 600 uL in each spin column, with 2 spin columns per eppie and 8 total spin columns.
11. Spin all spin columns at 1500 rpm for one minute.
12. Label four cuvettes A, B, C, and D. Combine all spin column flow through liquid originating from the same eppie into the corresponding cuvette.
13. Measure OD of all four liquid samples in the cuvettes.

Summary Table

	Liquid Culture Volume (uL)	5% BSA in PBS Volume (uL)	Antibody	Antibody Volume (uL)	Antibody Dilution	OD_o	OD_f
A	600	600	none	0	0		
B	600	600	specific	6	1:200		
C	600	600	specific	0.5	1:2400		
D	600	600	non-specific	6	1:200		